CLAIMS

1. A method of determining the presence of anti-Factor VIII allo-antibodies capable of degrading Factor VIII in a mammal, characterised in that it comprises :

i) isolating the plasma from a sample of blood taken from said mammal,

ii) isolating anti-Factor VIII allo-antibodies from said plasma ;

iii) placing said anti-Factor VIII allo-antibodies in contact with Factor VIII for a period of time sufficient to permit any degradation of said Factor VIII by said anti-Factor VIII allo-antibodies ; and

iv) determining, after said period of time, whether said Factor VIII has effectively been degraded by said anti-Factor VIII allo-antibodies.

2. The method according to claim 1, characterised in that in step ii), said anti-Factor VIII allo-antibodies are isolated from said plasma by combining them with said Factor VIII, said Factor VIII being preferably coupled to a matrix.

3. The method according to claim 1 or 2, characterised in that in step ii), said anti-Factor VIII allo-antibodies are isolated by affinity chromatography.

4. The method according to claim 3, characterised in that step ii), said affinity chromatography comprises the use of Factor VIII covalently coupled to a Sepharose matrix, preferably activated with cyanogen bromide.

5. The method according to any one of claims 1 to 4, characterised in that in step iii), said Factor VIII is labelled with a labelling agent, preferably a radio-labelling agent, such as ^{125}I in particular.

6. The method according to any one of claims 1 to 5, characterised in that in step iii), said Factor VIII is placed in contact with the anti-Factor VIII allo-antibodies for a period of time of between about 0.5 and about 30 hours, preferably about 10 hours, at a temperature of about 15 to about 40°C, preferably 38°C.

7. The method according to any one of claims 1 to 6, characterised in that step iv) is carried out by a determination comprising a separation technique, such as gel electrophoresis, such as SDS PAGE in particular, or gel filtration, such as fast protein liquid chromatography gel filtration in particular, and a visualisation technique, such as autoradiography in particular.

8. The method according to any one of claims 1 to 7, characterised in that it further comprises :

v) characterising the site(s) in said Factor VIII molecule cleaved by said anti-Factor VIII allo-antibodies.

9. The method according to claim 8, characterised in that said characterisation is carried out by placing said Factor VIII in contact with said anti-Factor VIII allo-antibodies capable of degrading Factor VIII, separating and then sequencing the fragments of Factor VIII resulting therefrom.

10. The method according to claim 8 or 9, characterised in that said separation is carried out using a technique such as gel electrophoresis, such as SDS PAGE in particular.

11. The method according to any one of claims 8 to 10, characterised in that said sequencing is carried out using a technique such as N-terminal sequencing, such as by using an automatic protein microsequencer in particular.

12. The method according to any one of claims 8 to 11, characterised in that said sequencing locates scissile bonds : Arg^{372}-Ser^{373}, located between the A1 and A2 domains, Tyr^{1680}-Asp^{1681} , located on the N-terminus of the A3 domain, and Glu^{1794}-Asp^{1795} located within the A3 domain of the Factor VIII molecule.

13. An amino acid sequence :

Ser Val Ala Lys Lys His Pro .

14. An amino acid sequence :

Asp Glu Asp Glu Asn Gln Ser .

15. An amino acid sequence :

Asp Gln Arg Gln Gly Ala Glu .

16. A peptide or non-peptide analogue of an amino acid sequence of any one of claims 13 to 15, characterised in that it is capable of inhibiting any site in the Factor VIII molecule which is susceptible to being lysed by an anti-Factor VIII allo-antibody.

17. An anti-Factor VIII allo-antibody-catalysed Factor VIII degradation inhibitor.

18. The inhibitor according to claim 17, characterised in that it comprises a protease inhibitor, such as 4-(2-aminoethyl)benzenesulphonyl fluoride hydrochloride in particular.

19. The inhibitor according to claim 17 or 18, characterised in that said inhibitor inhibits cleavage of the scissile bonds : Arg^{372}-Ser^{373}, located between the A1 domains, Tyr^{1680}-Asp^{1681} , located on the N-terminus of the A3 domain, and Glu^{1794}-Asp^{1795} located within the A3 domain of the Factor VIII molecule.

20. The inhibitor according to any one of claims 17 to 19, characterised in that it comprises a peptide or non-peptide analogue of the amino acid sequence :

Ser Val Ala Lys Lys His Pro .

21. The inhibitor according to any one of claims 17 to 19, characterised in that it comprises a peptide or non-peptide analogue of the amino acid sequence :

Asp Glu Asp Glu Asn Gln Ser .

22. The inhibitor according to any one of claims 17 to 19, characterised in that it comprises a peptide or non-peptide analogue of the amino acid sequence :

Asp Gln Arg Gln Gly Ala Glu .

23. A pharmaceutical composition characterised in that it comprises a pharmaceutically effective amount of an anti-Factor VIII allo-

antibody capable of degrading Factor VIII, notably as obtainable from the method of any one of claims 1 to 6.

24. Use of anti-Factor VIII allo-antibodies capable of degrading Factor VIII for the preparation of a pharmaceutical composition for the treatment of a mammal suffering from a pathology resulting from abnormal level of Factor VIII in the blood thereof.

25. Use according to claim 24, characterised in that said pathology results from the presence of an excess of Factor VIII in the blood thereof.

26. A pharmaceutical composition characterised in that it comprises a pharmaceutically effective amount of a Factor VIII degradation inhibitor according to any one of claims 17 to 22.

27. Use of a Factor VIII degradation inhibitor for the preparation of a pharmaceutical composition, in particular for the treatment of a mammal suffering from a pathology resulting from the sub-physiological level of Factor VIII in the blood thereof.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 February 2001 (01.02.2001)

PCT

(10) International Publication Number
WO 01/07918 A1

(51) International Patent Classification[7]: G01N 33/68, C07K 7/06, A61K 38/08, 39/395

(21) International Application Number: PCT/EP00/06870

(22) International Filing Date: 18 July 2000 (18.07.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
99401841.4 21 July 1999 (21.07.1999) EP

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/07918 A1

(54) Title: CATALYTIC ANTI-FACTOR VIII ALLO-ANTIBODIES

(57) Abstract: The present invention relates to a method of determining the presence of catalytic anti-Factor VIII allo-antibodies capable of degrading Factor VIII in a mammal, and of characterising the cleavage sites in said Factor VIII molecule by said catalytic anti-Factor VIII allo-antibodies. It also relates to an anti-Factor VIII allo-antibody-catalysed Factor VIII degradation inhibitor; and to a pharmaceutical composition comprising said catalytic anti-Factor VIII allo-antibodies which are capable of degrading Factor VIII and which originate from said method of determination; and further to a pharmaceutical composition comprising said anti-Factor VIII allo-antibody-catalysed Factor VIII degradation inhibitor. Finally, the present invention relates to the application in therapeutics of said anti-Factor VIII allo-antibody-catalysed Factor VIII degradation inhibitor, of a pharmaceutical composition comprising said catalytic anti-Factor VIII allo-antibodies which are capable of degrading Factor VIII and which originate from said method of determination, and of a pharmaceutical composition comprising said anti-Factor VIII allo-antibody-catalysed Factor VIII degradation inhibitor.

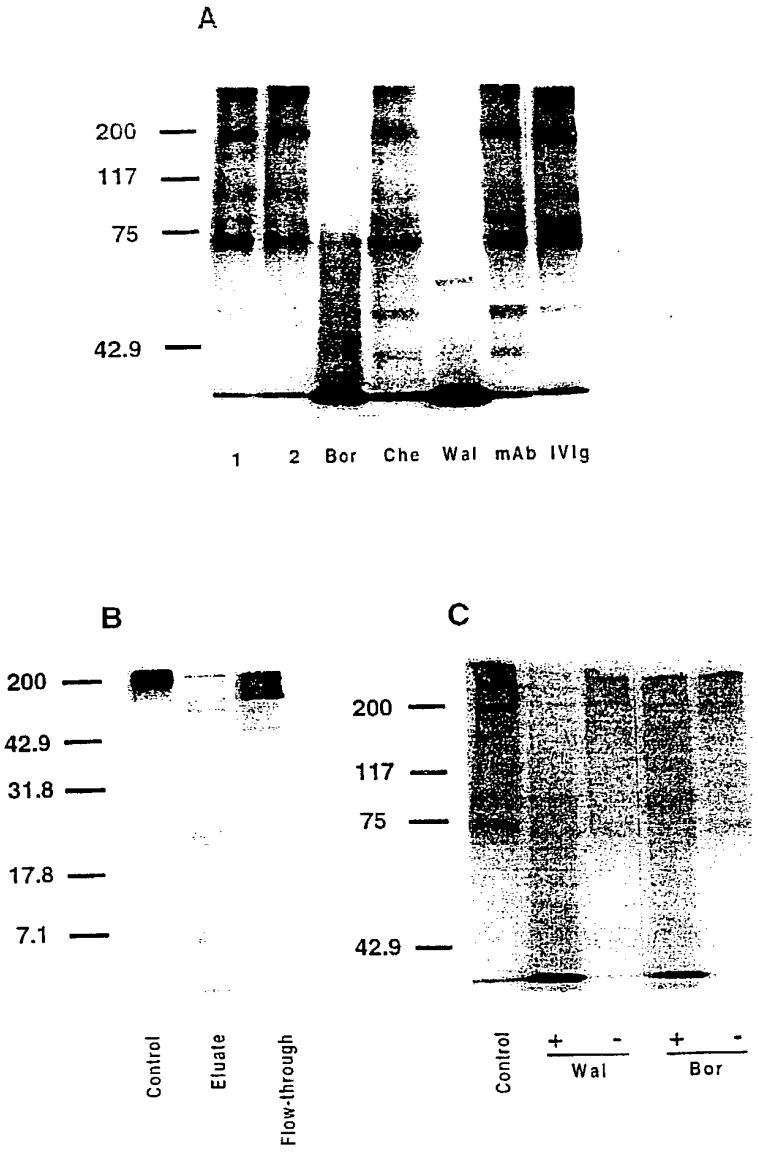

A
200
117
75
42.9
1
2
Bor
Che
Wal
mAb
IVIg
B
200
42.9
31.8
17.8
7.1
Control
Eluate
Flow-through
C
200
117
75
42.9
Control
+
-
Wal
+
-
Bor

FIG.1

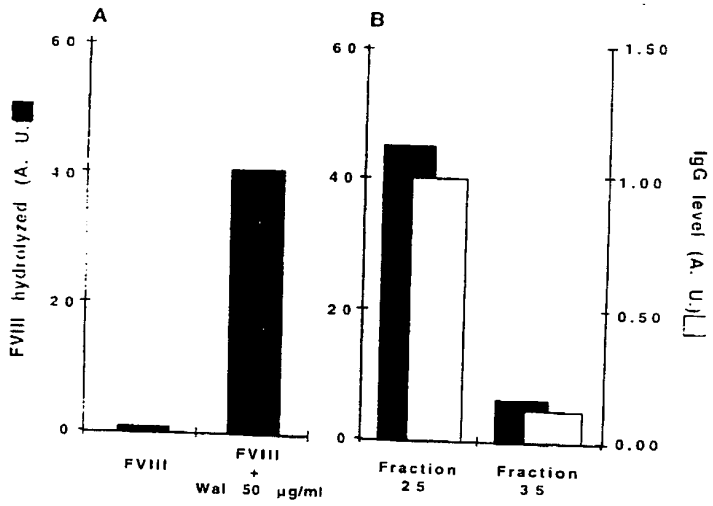

A
B
60
40
20
0
FVIII hydrolyzed (A. U.)
FVIII
FVIII + Wal 50 µg/ml
Fraction 25
Fraction 35
1.50
1.00
0.50
0.00
IgG level (A. U.)

FIG. 2

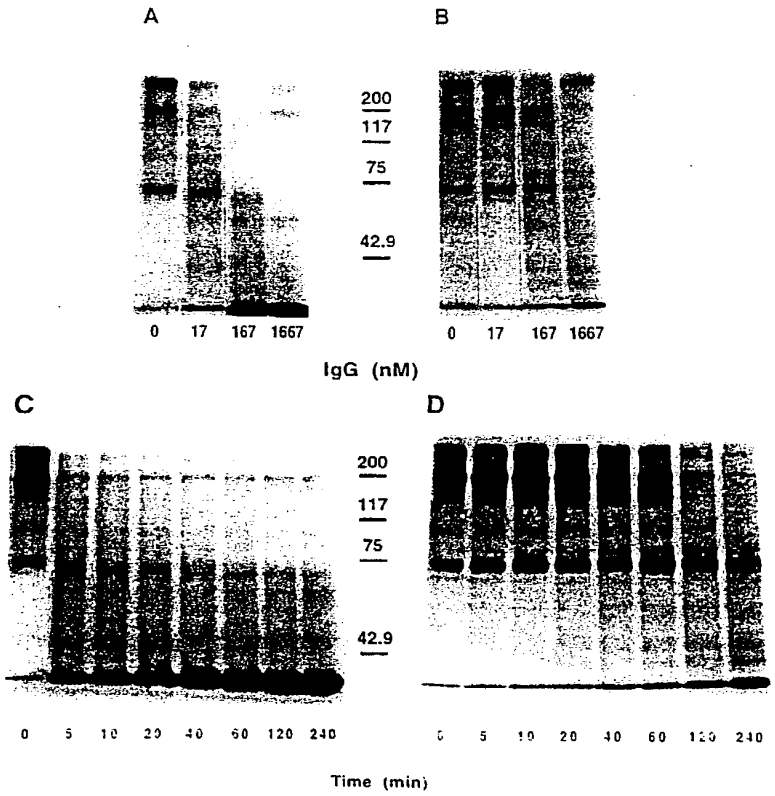
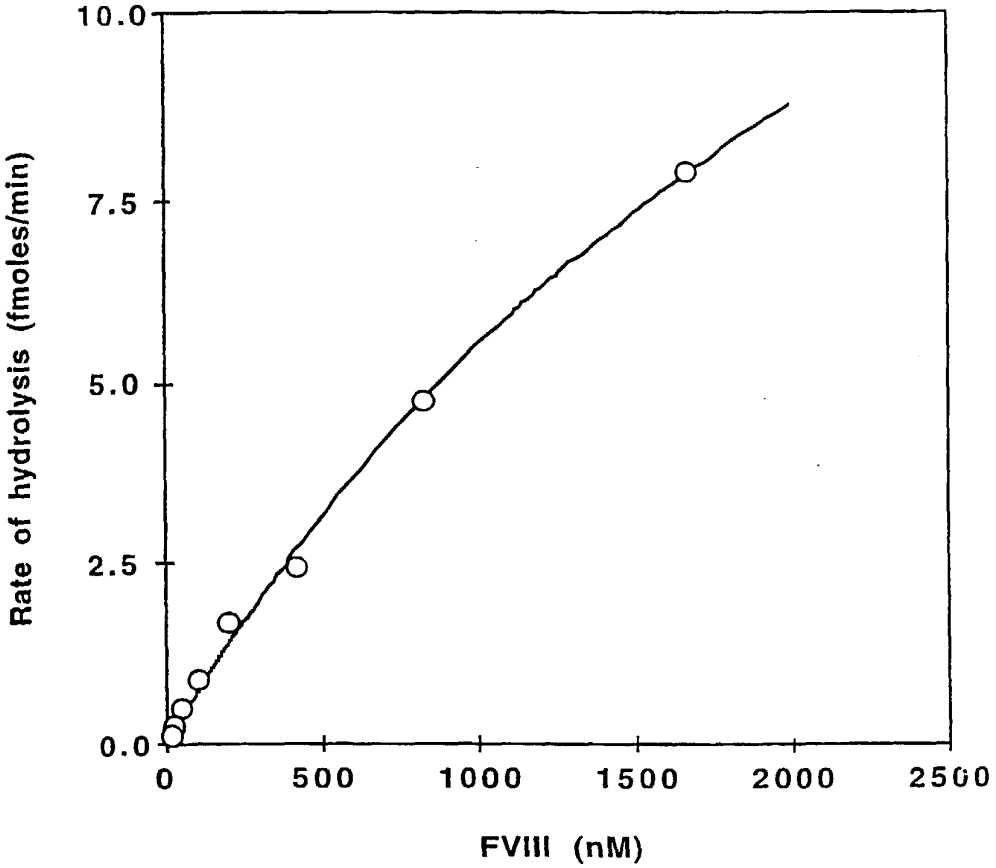

A
B
200
117
75
42.9
0 17 167 1667
0 17 167 1667
IgG (nM)
C
D
200
117
75
42.9
0 5 10 20 40 60 120 240
0 5 10 20 40 60 120 240
Time (min)

FIG. 3



K_m (μM)	9.46 ± 5.62
V_{Max} (fmoles/min)	85.1 ± 60.1
Catalytic constant (min^{-1})	0.026 ± 0.018
Catalytic efficiency $(M.min)^{-1}$	2553 ± 533

FIG. 4

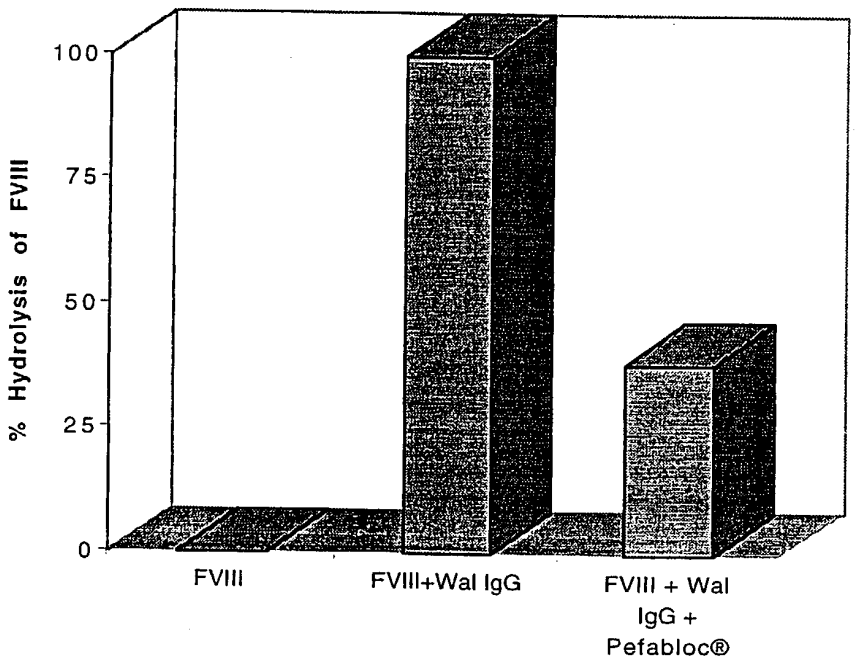

% Hydrolysis of FVIII
100
75
50
25
0
FVIII
FVIII+Wal IgG
FVIII + Wal IgG + Pefabloc®

FIG. 5